

Short Communication

Effect of *Eucalyptus* extract against *E. coli* O104:H4 in the minced meat of trout**Authors:****Fatemeh Dadmarzi****Institution:**MSc in Food Industry
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ABSTRACT:

One of the most important bacteria which is transmitted through the use of marine products, is *E. coli* O104:H4 bacteria. The main reason for this contamination is the poor maintenance of water at unhealthy conditions. Therefore, the use of a preservative with antibacterial property in marine products is essential. Hence, in the present study, the antibacterial effect of hydro-methanolic extract of *Eucalyptus* against *E. coli* in the trout minced meat during the storage period in the refrigerator was investigated. The behaviour of *Escherichia coli* at 500 and 1000 ppm of *Eucalyptus* extract were noted. It was found that *Eucalyptus* extract at a concentration of 1000 ppm could reduce the growth rate of *E. coli* bacteria below the allowed limit from the 8th day until the end of the maintenance period. According to the results, *Eucalyptus* hydro-methanolic extract with a concentration of 1000 ppm can be used as a natural anti-bacterial agent in the fishery products.

Keywords:*Eucalyptus*, Hydro-methanolic, Minced meat, trout, *E. coli*.**Article Citation:****Fatemeh Dadmarzi**Effect of *Eucalyptus* extract against *E. coli* O104:H4 in the minced meat of trout
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INTRODUCTION

Because of having high quality protein, high unsaturated fats, vitamins and minerals aquatic organisms are considered as good food for health (Javadiyan, 2011). Basically, *E. coli* is a common cause of food poisoning. The bacteria enters through the mouth, and they stick to the cells of the intestinal mucus. This causes intestinal mucus to be destroyed and subsequently resulting in severe abdominal pain and diarrhea. Minimum pathogenicity is 10^{10} /g of food (Ehsan *et al.*, 2010). *Escherichia coli* is a gram negative bacteria belonging to Enterobacteriaceae family (Madigan and Martinko, 2006). The increase in the intestinal pathogenic bacteria is an important factor leading to diarrhea and gastrointestinal disorders. This bacterium is also a natural flora of the large intestines of warm blooded animals. The presence of this microorganism in food represents a fecal contamination (Eslamo *et al.*, 2009).

The *Eucalyptus* tree is native to Australia, but now has spread to many other parts of the world. The word *Eucalyptus* was derived from two Greek words 'eu' meaning good and 'kalypso' meaning hidden. This tree was introduced in Iran about half a century ago at different regions, especially in the south of the country, which is suitable for its growth. In traditional medicine, *Eucalyptus* is used orally for the treatment of bladder/ fever/ pertussis/ low appetite and is used topically to treat wounds/ acne/ bleeding gums and rheumatism (Oyedegi *et al.*, 1999).

MATERIALS AND METHODS

10 kg of trout were procured from a fishmonger in market. The fish were washed repeatedly with water, and the heads, tails and fins were removed. After abdominal drainage by washing again, skinning was done, and it was rotated twice by a meat grinder. The minced meat thus prepared was then maintained below 4°C until the time of bacterial isolation at the refrigerator (Abdullahzadeh *et al.*, 2011).

Culture of *E. coli*

Standard strain of the bacteria *E. coli* O104:H4 was obtained from the Organization of Scientific and Industrial Research of Iran. The lyophilized capsules containing bacteria were first opened at sterile conditions and then transferred to a liquid culture medium TSB (Tryptic Soy Broth) and incubated at 48°C for 48h. Afterwards, the medium containing bacteria was centrifuged for five minutes. The supernatant was replaced with a ringer solution. In order to completely isolate the culture medium from the resulting bacteria, it was again centrifuged for five minutes. The number of bacteria in the underlying fluid was obtained by turbidity method at a wavelength of 570 nm, so that the optical absorption of 0.08 to 0.1 was considered to be approximately 10^8 bacteria per ml. In order to confirm the results, bacterial count by the surface cultivation on a medium containing Muller Hinton agar was done (Eloff, 1998).

Impregnation of sample in the extract and inoculation with the *E. coli*

Methanolic extract of *Eucalyptus* was obtained from Barij company (Isfahan, Iran). Minced meat samples were inoculated with 10^{1-4} CFU/g bacteria. (Babayi *et al.*, 2004). Subsequently, the inoculated meat were completely homogenized. Samples were immersed at various levels (500 and 1000 ppm) of hydro-methanolic extract of *Eucalyptus* for experimentation purpose. For each treatment, seven packets of five grams of meat were considered. All treatments were packed in zipped nylon bags and kept at the refrigerator during the 12 days of experiment. *E. coli* count was done at every 48h using Solomakos *et al.* (2008) method. In total, the current study include three treatments, control sample without preservative, specimen containing 500 ppm of *Eucalyptus* hydro-methanolic extract, and the sample containing 1000 ppm of *Eucalyptus* hydro-methanolic extract.

Counting *E. coli* bacteria

CHROMagar ECC culture medium was used to

count *E. coli*. At each sampling time, 5g of sample meat was added to 45ml of physiological serum and then homogenized. The dilution of the sample varied from 10^{-2} to 10^{-4} . 0.1ml diluted sample was cultured on CHRO-Magar ECC medium and incubated at 44°C for 24h. For each treatment, three replicates were considered and the colonies were counted (Buller, 2004).

Data analysis

Normalization of data homogeneity of variance and one-way ANOVA were used to analyze the variance. Duncan test was used at 5% level to compare the data. All data was reported in the form of mean \pm SD. SPSS version 18 software (SPSS, 2009) was used for analyzing the data and MS excel was used to draw the graphical representations.

RESULTS AND DISCUSSION

The results for different treatments are shown in Figure 1. In general, changes over time in the control treatment increased significantly ($P < 0.05$). But in the treatment of 500 ppm *Eucalyptus* extract, upto six days the concentration of *E. coli* increased and then it decreased. And at 1000 ppm *Eucalyptus* extract concentration, the growth of *E. coli* increased for two days and

then decreased. Comparison of treatments, during different maintenance periods indicated that *Eucalyptus* extract at a concentration of 1000 ppm showed significantly more antagonistic than at the concentration of 500 ppm than in the control ($P < 0.05$). The use of *Eucalyptus* extract at different concentrations showed that 500 ppm for six days showed more effect than the 1000 ppm for four days, The amount of *E. coli* bacteria has considerably been decreased and the standards, were maintained in the *E. coli* bacteria limit in meat. The treatment at eight days considerably reduced the bacteria, that is, 98.1 log cfu/g, whose amount in this treatment reached 12.27 log cfu/g, at the 12th day. These changes indicated that *Eucalyptus* extract with a concentration of 1000 ppm after 8 days, inhibits the growth of *E. coli* bacteria and destroys it. The amount of it transmitted an acceptable limit of *E. coli* bacteria. Studies carried out by Friedman *et al.* (2002) and Marino *et al.* (2007) have also confirmed that herbal extracts had antibacterial effect. Several studies have reported that the effect of antibacterial essential oil and herbal extracts are dependent on the dose they intake (Burt, 2004). The results of this study are also consistent with the results of Noori *et al.* (2012) who investigated the

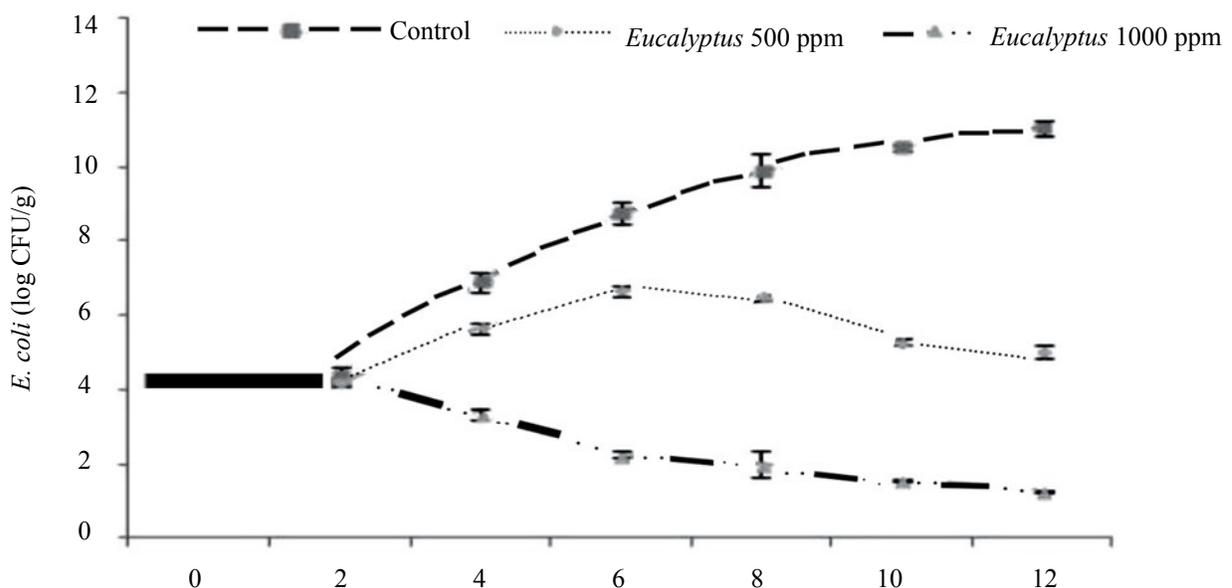


Figure 1. *E. coli* concentration at different treatments during the storage

antimicrobial effect of thyme oil at different concentrations on *E. coli* in the minced meat of the calf, during the storage period at the refrigerator temperature. Behbahani *et al.* (2012) by examining the amount of *E. coli* in common minced fish meat, containing the ethanolic extract of *Eucalyptus* leaf, stated that the extract has a natural preservative and reduce the microbial load in foodstuffs, including aquatic animals. Kukric *et al.* (2012) in the study of antimicrobial activity of *Urtica* leaf extract expressed that its extract was able to reduce the gram positive and negative bacteria.

Failure to use proper fish keeping techniques and marine habitats have led to the rapid changes at various microbiological and sensory factors and has resulted in the fish contamination. Also, bacterial contamination, including *E. coli* bacteria, in fish, especially sliced fish meat is harmful and natural preservatives with antimicrobial and antibacterial properties were used. Meanwhile, the *Eucalyptus* extract has good antibacterial properties, therefore, in this research, we prepared treatments with antibacterial properties of *Eucalyptus* extract at different concentrations and it was used for the maintenance of minced meat. Also, the antibacterial effect against *E. coli* on cold storage was investigated. The results of microbial analysis showed that the extracts containing concentrations of 1000 ppm could inhibit the growth of *E. coli* bacteria.

CONCLUSION

In general, the results of the present study confirmed the use of *Eucalyptus* extract with a concentration of 1000 ppm would aid in preserving the quality and increasing the shelf life of the minced meat.

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